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(54) Title: IDENTIFICATION OF BIOLOGICAL (MICRO) ORGANISMS BY DETECTION OF THEIR HOMOLOGOUS NUCLEOTIDE SEQUENCES ON ARRAYS

(57) Abstract: The present invention is related to an identification and/or quantification method of a biological (micro)organism or part of it by a detection of its nucleotide sequence among at least 4 other homologous sequences and comprising: amplifying or copying with a unique pair of primer(s), at least part of original nucleotide sequences (1) into target nucleotide sequences (2) to be detected; possibly labelling said target nucleotide sequences (2); putting into contact the labelled target nucleotide sequences (2) with single stranded capture nucleotide sequences (3) bound by a single predetermined link to an insoluble solid support (4), preferably a non porous solid support, discriminating the binding of a target nucleotide sequence (2) specific of an organism or part of it by detecting, quantifying and/or recording a signal resulting from a hybridization by complementary base pairing between the target nucleotide sequence (2) and its corresponding capture nucleotide sequence (3), wherein said capture nucleotide sequence (3) being bound to the insoluble solid support (4) at a determined location according to an array, said array having a density of at least 4 different bound single stranded capture nucleotide sequences/cm2 of solid support surface.

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International Application No
PCT/BE 01/00053

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, BIOSIS, EMBASE, MEDLINE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X Y	WO 97 29212 A (GINGERAS THOMAS A ;CHEE MARK S (US); STRYER LUBERT (US); AFFYMETRI) 14 August 1997 (1997-08-14) the whole document	1-14,16, 24,25, 27,28 15,17, 26,29

X Y	WO 98 28444 A (UNIV CHICAGO) 2 July 1998 (1998-07-02) the whole document	1-14,16, 24,25, 27,28 15,17, 26,29

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 January 2002	10. 04. 2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hagenmaier, S

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GUO Z ET AL: "DIRECT FLUORESCENCE ANALYSIS OF GENETIC POLYMORPHISMS BY HYBRIDIZATION WITH OLIGONUCLEOTIDE ARRAYS ON GLASS SUPPORTS" NUCLEIC ACIDS RESEARCH,GB,OXFORD UNIVERSITY PRESS, SURREY, vol. 22, no. 24, 11 December 1994 (1994-12-11), pages 5456-5465, XP002006248 ISSN: 0305-1048	1-14,16, 24,25
Y	the whole document	15,17, 26-29

Y	WO 99 16780 A (GALA JEAN LUC ;UNIV LOUVAIN (BE); MINISTERE DE LA DEFENSE NATION ()) 8 April 1999 (1999-04-08) the whole document	1-17, 24-29

Y	VANNUFFLE ET AL.: "Combined discrimination between Staphylococcus species and identification of methicillin resistance by a sandwich enzyme-linked oligo sorbent assay" ABSTRACTS OF THE INTERSCIENCE CONFERENCE ON ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, vol. 39, 29 September 1999 (1999-09-29), page 208 XP001053081 the whole document	1-17, 24-29

Y	WO 89 11548 A (CETUS CORP) 30 November 1989 (1989-11-30) the whole document	1-17, 24-29

Y	EP 0 511 559 A (HOFFMANN LA ROCHE) 4 November 1992 (1992-11-04) the whole document	1-17, 24-29

Y	GB 2 318 791 A (ZENECA LTD) 6 May 1998 (1998-05-06) the whole document	1-17, 24-29

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/BE 01/00053

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 98 11253 A (ERNEST ISABELLE ;REMACLE JOSE (BE); ALEXANDRE ISABELLE (BE); ZAMMA) 19 March 1998 (1998-03-19) the whole document	

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/BE 01/00053

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-16, 24-28 (all partially), 17, 29 (completely)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-16, 24-28 (all partially), 17,29 (completely)

Diagnostic kit and method for the identification and/or quantification of a biological (micro) organism or part of it (possibly present in a biological sample) by a detection of its nucleotide sequence among at least 4 other homologous sequences and comprising the steps of :
-possibly extracting original nucleotide sequences (1) from the (micro) organism ;
-amplifying or copying with a unique pair of primer(s), at least part of original nucleotide sequences (1) into target nucleotide sequences (2) to be detected ;
-possibly labelling said target nucleotide sequences (2) ;
-putting into contact the labelled target nucleotide sequences (2) with single stranded capture nucleotide sequences (3) bound by a single predetermined link to an insoluble solid support (4), preferably a non porous solid support,
-discriminating the binding of a target nucleotide sequence (2) specific of an organism or part of it by detecting, quantifying and/or recording a signal resulting from a hybridization by complementary base pairing between the target nucleotide sequence (2) and its corresponding capture nucleotide sequence (3), wherein said capture nucleotide sequence (3) being bound to the insoluble solid support (4) at a determined location according to an array, said array having a density of at least 4 different bound single stranded capture nucleotide sequences/cm2 of solid support surface and wherein the binding between the target nucleotide sequence and its corresponding capture nucleotide sequence forms (will result in) said signal at determined location, the detection of a single signal allowing a discrimination and identification of the target nucleotide sequence specific of an organism or part of it from homologous nucleotide sequences wherein the solid support bears capture nucleotide sequences specific for the identification of two or more Staphylococcus species together with a consensus sequence for a Staphylococcus genus identification.

2. Claims: 1-16, 24-28 (all partially), 18,30 (completely)

Diagnostic kit and method for the identification and/or quantification of a biological (micro) organism or part of it (possibly present in a biological sample) by a detection of its nucleotide sequence among at least 4 other homologous sequences and comprising the steps of :
-possibly extracting original nucleotide sequences (1) from the (micro) organism ;
-amplifying or copying with a unique pair of primer(s), at least part of original nucleotide sequences (1) into target

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

nucleotide sequences (2) to be detected ;
-possibly labelling said target nucleotide sequences (2) ;
-putting into contact the labelled target nucleotide sequences (2) with single stranded capture nucleotide sequences (3) bound by a single predetermined link to an insoluble solid support (4), preferably a non porous solid support,
-discriminating the binding of a target nucleotide sequence (2) specific of an organism or part of it by detecting, quantifying and/or recording a signal resulting from a hybridization by complementary base pairing between the target nucleotide sequence (2) and its corresponding capture nucleotide sequence (3), wherein said capture nucleotide sequence (3) being bound to the insoluble solid support (4) at a determined location according to an array, said array having a density of at least 4 different bound single stranded capture nucleotide sequences/cm2 of solid support surface and wherein the binding between the target nucleotide sequence and its corresponding capture nucleotide sequence forms (will result in) said signal at determined location, the detection of a single signal allowing a discrimination and identification of the target nucleotide sequence specific of an organism or part of it from homologous nucleotide sequences wherein the original sequence to be identified and/or quantified in the sample belongs to the MAGE gene family.

3. Claims: 1-16, 24-28 (all partially), 19,31 (completely)

Diagnostic kit and method for the identification and/or quantification of a biological (micro) organism or part of it (possibly present in a biological sample) by a detection of its nucleotide sequence among at least 4 other homologous sequences and comprising the steps of :
-possibly extracting original nucleotide sequences (1) from the (micro) organism ;
-amplifying or copying with a unique pair of primer(s), at least part of original nucleotide sequences (1) into target nucleotide sequences (2) to be detected ;
-possibly labelling said target nucleotide sequences (2) ;
-putting into contact the labelled target nucleotide sequences (2) with single stranded capture nucleotide sequences (3) bound by a single predetermined link to an insoluble solid support (4), preferably a non porous solid support,
-discriminating the binding of a target nucleotide sequence (2) specific of an organism or part of it by detecting, quantifying and/or recording a signal resulting from a hybridization by complementary base pairing between the target nucleotide sequence (2) and its corresponding capture nucleotide sequence (3), wherein said capture nucleotide sequence (3) being bound to the insoluble solid support (4) at a determined location according to an array, said array having a density of at least 4 different bound single

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

stranded capture nucleotide sequences/cm2 of solid support surface and wherein the binding between the target nucleotide sequence and its corresponding capture nucleotide sequence forms (will result in) said signal at determined location, the detection of a single signal allowing a discrimination and identification of the target nucleotide sequence specific of an organism or part of it from homologous nucleotide sequences wherein the original sequence to be identified and/or quantified in the sample belongs to the HLA-A genes family.

4. Claims: 1-16, 24-28,32 (all partially), 20,33 (completely)

Diagnostic kit and method for the identification and/or quantification of a biological (micro) organism or part of it (possibly present in a biological sample) by a detection of its nucleotide sequence among at least 4 other homologous sequences and comprising the steps of :
-possibly extracting original nucleotide sequences (1) from the (micro) organism ;
-amplifying or copying with a unique pair of primer(s), at least part of original nucleotide sequences (1) into target nucleotide sequences (2) to be detected ;
-possibly labelling said target nucleotide sequences (2) ;
-putting into contact the labelled target nucleotide sequences (2) with single stranded capture nucleotide sequences (3) bound by a single predetermined link to an insoluble solid support (4), preferably a non porous solid support,
-discriminating the binding of a target nucleotide sequence (2) specific of an organism or part of it by detecting, quantifying and/or recording a signal resulting from a hybridization by complementary base pairing between the target nucleotide sequence (2) and its corresponding capture nucleotide sequence (3), wherein said capture nucleotide sequence (3) being bound to the insoluble solid support (4) at a determined location according to an array, said array having a density of at least 4 different bound single stranded capture nucleotide sequences/cm2 of solid support surface and wherein the binding between the target nucleotide sequence and its corresponding capture nucleotide sequence forms (will result in) said signal at determined location, the detection of a single signal allowing a discrimination and identification of the target nucleotide sequence specific of an organism or part of it from homologous nucleotide sequences wherein the original sequence to be identified and/or quantified in the sample belongs to the dopamine receptors coupled to the protein G genes family.

5. Claims: 1-16, 24-28 (all partially), 21 (completely)

Diagnostic kit and method for the identification and/or

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

quantification of a biological (micro) organism or part of it (possibly present in a biological sample) by a detection of its nucleotide sequence among at least 4 other homologous sequences and comprising the steps of :
-possibly extracting original nucleotide sequences (1) from the (micro) organism ;
-amplifying or copying with a unique pair of primer(s), at least part of original nucleotide sequences (1) into target nucleotide sequences (2) to be detected ;
-possibly labelling said target nucleotide sequences (2) ;
-putting into contact the labelled target nucleotide sequences (2) with single stranded capture nucleotide sequences (3) bound by a single predetermined link to an insoluble solid support (4), preferably a non porous solid support,
-discriminating the binding of a target nucleotide sequence (2) specific of an organism or part of it by detecting, quantifying and/or recording a signal resulting from a hybridization by complementary base pairing between the target nucleotide sequence (2) and its corresponding capture nucleotide sequence (3), wherein said capture nucleotide sequence (3) being bound to the insoluble solid support (4) at a determined location according to an array, said array having a density of at least 4 different bound single stranded capture nucleotide sequences/cm2 of solid support surface and wherein the binding between the target nucleotide sequence and its corresponding capture nucleotide sequence forms (will result in) said signal at determined location, the detection of a single signal allowing a discrimination and identification of the target nucleotide sequence specific of an organism or part of it from homologous nucleotide sequences wherein the original sequence to be identified and/or quantified in the sample belongs to the choline receptors coupled to the protein G genes family.

6. Claims: 1-16, 24-28,32 (all partially),22,35 (completely)

Diagnostic kit and method for the identification and/or quantification of a biological (micro) organism or part of it (possibly present in a biological sample) by a detection of its nucleotide sequence among at least 4 other homologous sequences and comprising the steps of :
-possibly extracting original nucleotide sequences (1) from the (micro) organism ;
-amplifying or copying with a unique pair of primer(s), at least part of original nucleotide sequences (1) into target nucleotide sequences (2) to be detected ;
-possibly labelling said target nucleotide sequences (2) ;
-putting into contact the labelled target nucleotide sequences (2) with single stranded capture nucleotide sequences (3) bound by a single predetermined link to an insoluble solid support (4), preferably a non porous solid support,

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-discriminating the binding of a target nucleotide sequence (2) specific of an organism or part of it by detecting, quantifying and/or recording a signal resulting from a hybridization by complementary base pairing between the target nucleotide sequence (2) and its corresponding capture nucleotide sequence (3), wherein said capture nucleotide sequence (3) being bound to the insoluble solid support (4) at a determined location according to an array, said array having a density of at least 4 different bound single stranded capture nucleotide sequences/cm2 of solid support surface and wherein the binding between the target nucleotide sequence and its corresponding capture nucleotide sequence forms (will result in) said signal at determined location, the detection of a single signal allowing a discrimination and identification of the target nucleotide sequence specific of an organism or part of it from homologous nucleotide sequences wherein the original sequence to be identified and/or quantified in the sample belongs to the histamine receptors coupled to the protein G genes family.

7. Claims: 1-16, 24-28 (all partially), 23,37 (completely)

Diagnostic kit and method for the identification and/or quantification of a biological (micro) organism or part of it (possibly present in a biological sample) by a detection of its nucleotide sequence among at least 4 other homologous sequences and comprising the steps of :
-possibly extracting original nucleotide sequences (1) from the (micro) organism ;
-amplifying or copying with a unique pair of primer(s), at least part of original nucleotide sequences (1) into target nucleotide sequences (2) to be detected ;
-possibly labelling said target nucleotide sequences (2) ;
-putting into contact the labelled target nucleotide sequences (2) with single stranded capture nucleotide sequences (3) bound by a single predetermined link to an insoluble solid support (4), preferably a non porous solid support,
-discriminating the binding of a target nucleotide sequence (2) specific of an organism or part of it by detecting, quantifying and/or recording a signal resulting from a hybridization by complementary base pairing between the target nucleotide sequence (2) and its corresponding capture nucleotide sequence (3), wherein said capture nucleotide sequence (3) being bound to the insoluble solid support (4) at a determined location according to an array, said array having a density of at least 4 different bound single stranded capture nucleotide sequences/cm2 of solid support surface and wherein the binding between the target nucleotide sequence and its corresponding capture nucleotide sequence forms (will result in) said signal at determined location, the detection of a single signal allowing a discrimination and identification of the target nucleotide

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sequence specific of an organism or part of it from homologous nucleotide sequences wherein the original sequence to be identified and/or quantified in the sample belongs to the cytochrome P450 forms family.

8. Claims: 24-25,27,28,32 (all partially), 34 (completely)

Diagnostic kit for performing a method for identification and/or quantification of a biological (micro) organism or part of it (possibly present in a biological sample) by a detection of its nucleotide sequence among at least 4 other homologous sequences and comprising the steps of :
-possibly extracting original nucleotide sequences (1) from the (micro) organism ;
-amplifying or copying with a unique pair of primer(s), at least part of original nucleotide sequences (1) into target nucleotide sequences (2) to be detected ;
-possibly labelling said target nucleotide sequences (2) ;
-putting into contact the labelled target nucleotide sequences (2) with single stranded capture nucleotide sequences (3) bound by a single predetermined link to an insoluble solid support (4), preferably a non porous solid support,
-discriminating the binding of a target nucleotide sequence (2) specific of an organism or part of it by detecting, quantifying and/or recording a signal resulting from a hybridization by complementary base pairing between the target nucleotide sequence (2) and its corresponding capture nucleotide sequence (3), wherein said capture nucleotide sequence (3) being bound to the insoluble solid support (4) at a determined location according to an array, said array having a density of at least 4 different bound single stranded capture nucleotide sequences/cm2 of solid support surface and wherein the binding between the target nucleotide sequence and its corresponding capture nucleotide sequence forms (will result in) said signal at determined location, the detection of a single signal allowing a discrimination and identification of the target nucleotide sequence specific of an organism or part of it from homologous nucleotide sequences wherein the original sequence to be identified and/or quantified in the sample belongs to the serotonine receptors coupled to the protein G genes family.

9. Claims: 24-28 (all partially), 36 (completely)

Diagnostic kit for performing a method for identification and/or quantification of a biological (micro) organism or part of it (possibly present in a biological sample) by a detection of its nucleotide sequence among at least 4 other homologous sequences and comprising the steps of :
-possibly extracting original nucleotide sequences (1) from the (micro) organism ;

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-amplifying or copying with a unique pair of primer(s), at least part of original nucleotide sequences (1) into target nucleotide sequences (2) to be detected ;
-possibly labelling said target nucleotide sequences (2) ;
-putting into contact the labelled target nucleotide sequences (2) with single stranded capture nucleotide sequences (3) bound by a single predetermined link to an insoluble solid support (4), preferably a non porous solid support,
-discriminating the binding of a target nucleotide sequence (2) specific of an organism or part of it by detecting, quantifying and/or recording a signal resulting from a hybridization by complementary base pairing between the target nucleotide sequence (2) and its corresponding capture nucleotide sequence (3), wherein said capture nucleotide sequence (3) being bound to the insoluble solid support (4) at a determined location according to an array, said array having a density of at least 4 different bound single stranded capture nucleotide sequences/cm2 of solid support surface and wherein the binding between the target nucleotide sequence and its corresponding capture nucleotide sequence forms (will result in) said signal at determined location, the detection of a single signal allowing a discrimination and identification of the target nucleotide sequence specific of an organism or part of it from homologous nucleotide sequences wherein the sequence to be identified and/or quantified in the sample are gene sequences of GMO plants.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/BE 01/00053

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